# New insight into biochemical methane production

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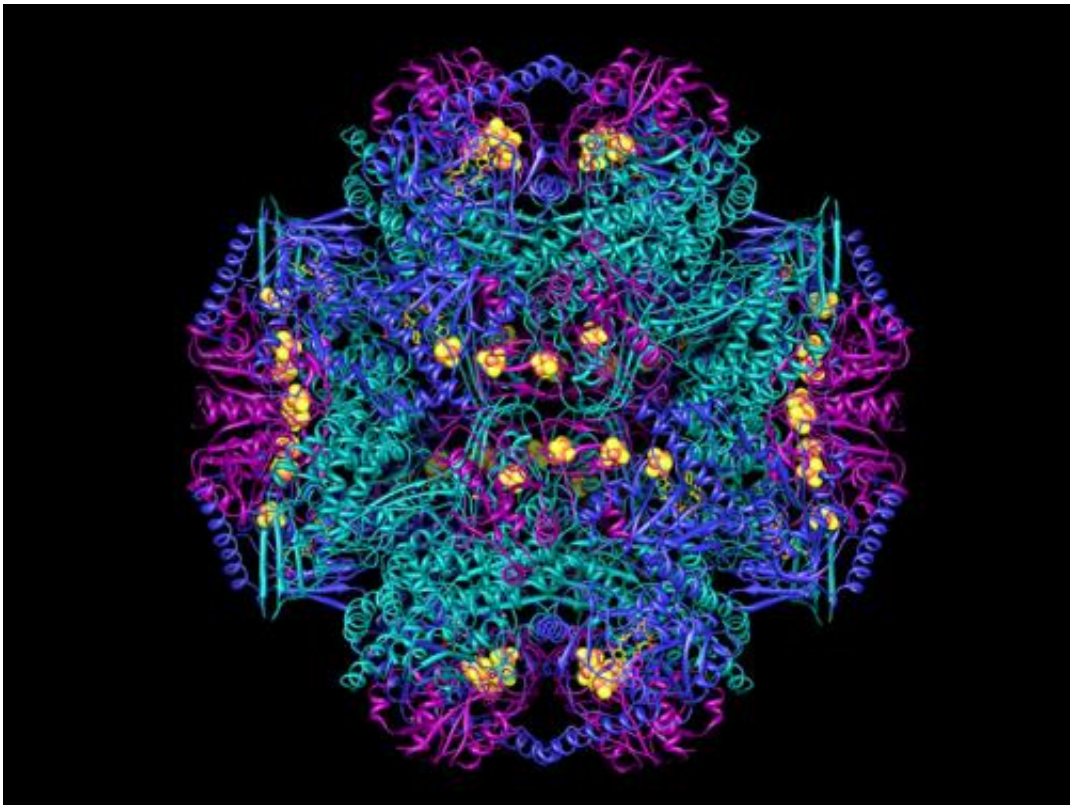

Molecular structure of the Frh proteins: one blue, one green and one violet subunit together form a trimer. Twelve of these trimers form the entire enzyme complex. The yellow structures show the reactive metal centres. Credit: MPI of Biophysics

The biological sources of methane are wide-ranging; however, the conditions have to be always oxygen-free. Archaebacteria release the

potent greenhouse gas in rice fields, mires and cows' stomachs, for example. A team of researchers from the Max Planck Institute of Biophysics in Frankfurt am Main and the Max Planck Institute for Terrestrial Microbiology in Marburg have gained insight into microbiological methane production by explaining the structure of the enzyme Frh using cryo-electron microscopy. This hydrogenase splits hydrogen so that it can be further processed for methane production from carbon dioxide. The Frankfurt-based scientists also identified the binding site for a coenzyme that is involved in other steps in the methane production process. A detailed understanding of the structure and functioning of hydrogenases could help in the development of synthetic catalysts for hydrogen production based on this biological model.

The extent to which cattle, rice fields and thawing permafrost soil are intensifying climate change is still undetermined. It is known, however, that archaebacteria in the intestines of cows and in oxygen-depleted water bodies and soil produce the highly potent greenhouse gas methane from hydrogen and carbon dioxide as well as from other carbon compounds. In order to assess the importance of biological methane production, scientists would like to understand the process in detail. Researchers at the Max Planck Institute of Biophysics in Frankfurt am Main and the Max Planck Institute for Terrestrial Microbiology in Marburg have contributed to this objective by discovering how the F420-reducing [NiFe]- hydrogenase, referred to as Frh, is structured, and the location of its docking site for the coenzyme F420.

Hydrogenases, which are found in the cell of archaebacteria, are important enzymes involved in the production of methane. They split hydrogen molecules into protons and electrons which are then transferred to carbon dioxide in a process involving several stages. To begin, Frh pushes the electrons towards the coenzyme F420, which makes them available for other reactions in the methanogenesis pathway. The enzyme belongs to the nickel-iron hydrogenases, in which the actual

reaction takes place in an active centre composed of nickel and iron. To date, the structure of the enzymes from only one of five groups into which the nickel-iron hydrogenases are divided, was known. Through the analysis of the structure of Frh, the Max Planck researchers have now discovered the structural details of another group of these biocatalysts.

"It was already known that Frh binds the coenzyme F420 in a special additional subunit," explains Janet Vonck, who carries out research at the Max Planck Institute of Biophysics. "However, we knew practically nothing about its detailed structure." The scientists have now decoded key aspects of the enzyme structure with the help of cryo-electron microscopy. With this method, the proteins are frozen in a thin layer of ice so that the biopolymers are immobilised. Using an electron microscope, the researchers then record numerous images of the proteins, which are present in different orientations. Then the images are combined computionally to calculate the three-dimensional structure of the complex.

## Hydrogenases as hydrogen producers

"We established that Frh forms large tetrahedral complexes," explains Vonck. Three different subunits form a functional unit. A total of twelve of these trimers combine to form a macromolecule. "We do not yet know why twelve trimers join forces," says Seigo Shima, a scientist at the Max Planck Institute for Terrestrial Microbiology and the Japan Science and Technology Agency. "The chemical reaction is also theoretically possible with just one of the trimers." The scientists also compared the structure of the enzyme without F420 with the structure to which the coenzyme was bound. In this way, they identified the pocket in which the coenzyme binds.

The scientists would now like to find out more about the structure and function of the hydrogenases, not only because this would enable them to

understand the influence of methane-producing archaebacteria on the climate better, but also because the detailed knowledge of the enzymes and their functioning could be of interest for technical applications. The reactions, in which hydrogenases split hydrogen into proteins and electrons, can also be reversed so that the enzymes produce hydrogen from protons and electrons. As soon as the links between the different blueprints and modes of functioning and the different characteristics of the hydrogenases are clear, the enzymes could be optimised for possible scientific uses. The most efficient hydrogenases that arise in nature are not stable enough and react very sensitively to oxygen in particular. If their blueprint could be altered in a way that would make them more robust, they could be made to produce hydrogen in technical systems using energy from sunlight.

**More information:** Mills, D. et al. De novo modelling of the F420-reducing [NiFe]-hydrogenase from a methanogenic archaeon by cryo-electron microscopy,
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